HEMATOCRIT AND ANALYTE CONCENTRATION DETERMINATION

The present invention relates to the field of hematocrit and analyte concentration determination. More precisely, the invention relates to a method to determine an analyte
5   concentration of an anticoagulated plasma by performing at least two different measurements on a mixture of a blood sample corresponding to said anticoagulated plasma and of liquid reagent. The invention also comprises a measurement and determination device for performing measurements on blood, anticoagulated blood and/or anticoagulated plasma samples, and an equipment kit.
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Background of the invention

As already mentioned, the subject invention concerns determination of analyte concentrations in anticoagulated plasma by measurements on mixtures of blood and liquid reagent. The results of such determinations are needed to make medical diagnosis and to
15   monitor the effects of medical treatments.

Determinations of analyte concentrations for medical purposes are traditionally performed at laboratories, distant from the patient. The results of the determinations are often needed at care facilities, close to the patient. This spatial situation creates a drive to perform analyte concentration determinations near the patient. Only anticoagulated blood
20   can be transported from care facilities to laboratories. At laboratories, the analyte determinations are performed on the anticoagulated blood or on anticoagulated plasma prepared from the anticoagulated blood. Since anticoagulated plasma is easier to work with and easier to store, laboratory determinations of analyte concentrations are performed on anticoagulated plasma, to the extent possible. At the near-patient facilities the situation is
25   different. Blood is readily available, but anticoagulated plasma is inconvenient or impossible to prepare. This gives rise to a situation where laboratory determinations of analyte concentrations are performed on anticoagulated plasma and near-patient determinations are performed on blood. The situation is unsatisfactory because rational medical practice requires the association between one analyte concentration value and one given patient at
30   one given time. Given the choice, clinicians would prefer the values of analyte concentration in anticoagulated plasma, because these values are association with a greater wealth of clinical reference data. Apart from the nature of the sample, the subject invention concerns accuracy and reliability of analyte concentration determinations. It is recognized, that accuracy and reliability are fundamental for the medical usefulness of analyte concentration
35   determinations.

Per definition, an analytical method is accurate if it generates results that are in agreement with those of a reference method. This also applies to near-patient methods to

determine analyte concentrations. A rational design strategy to obtain an accurate near-patient method is to adopt the chemistry and the assay conditions of a reference method, or of a method proven to be accurate according to the above. This straightforward strategy is difficult to follow. Reference methods are laboratory methods that represent culminations of

5   long-term collaborative researchers' efforts in laboratory milieus. These milieus and milieus of clinical laboratories are relatively similar. Assay condition that can be accomplished in one of these milieus can be accomplished in the other. The milieus within which near-patient concentration determinations are performed are markedly different. Already the first procedural step of a typical laboratory method, to mix a precise volume of anticoagulated

10   plasma and a precise volume of a reagent, represents a near insurmountable hurdle at near-patient assay sites. At surgical theaters, primary care centers, doctors' offices and patients' homes, anticoagulated plasma is inconvenient to prepare, and precise volumes are difficult to accomplish. Therefore, the first procedural step of a near-patient method is typically; to mix an imprecisely defined volume of blood with a dry reagent. The designers of near-patient

15   assay methods have not purposefully deviate from the assay conditions of the accurate laboratory methods; it has been forced upon them. Still, the deviations from laboratory assay conditions have inflicted accuracy flaws in the near-patient assay methods. This has caused concern and insecurity, and has compromised the safety and efficacy of medical diagnosis and treatments. A prudent strategy to improve the accuracy of near-patient analyte

20   concentration determinations is therefore, 1) to identify the aspects of assay conditions of laboratory methods that promote accuracy, and 2) to persistently adhere to the identified aspects in design of near patient methods.

As stated above, reference methods, and accurate laboratory methods, are often wet-chemistry methods. A main reason for the success of the wet-chemistry methods is

25   their universal potency in combating matrix effects. Mixing a small volume of sample with a large volume of reagent dilutes the sample. This diminishes all effects of the sample and sets the scene for assay conditions that selectively favor the effects of the analyte. The effects of the non-analytes of the sample, the matrix effects, are thereby disfavored and the accuracy of the assay enhanced.

30   Quantitative determination of analyte concentrations by wet-chemistry methods requires precise allotment of intended volumes, precisely what is difficult to accomplish at near-patient assay sites. The allotment may be well be precise, but in void of systems for checking volumetric equipment, properly trained laboratory personnel and other aspects of a well managed laboratory, the allotments of volumes at near-patient sites are bound to be

35   inaccurate, e.g. differ unacceptably from was intended. Prior art approaches to solving the problem is to invent 'user friendly', inexpensive, precise and accurate volumetric devices with

which near-patient methods may be practiced. Such approaches have experienced limited success.

Apart from the classical near-patient assay site mentioned above, near-patient assays are also performed at smaller laboratories and at divisions of larger laboratories. All near-patient assay sites share the aversion of preparing anticoagulated plasma but display a difference in their ability to accomplish precisely defined volumes. In the following, a distinction is made between near-patient assay sites and smaller laboratories. They share a preference for blood but differ in their ability to precisely allot intended volumes of blood and reagents.

Smaller laboratories and near-patient assay sites also share concerns regarding the reliability of analyte concentration determinations. Larger laboratories set the reliability standard. At larger laboratories, thousands of analyte concentrations per year, of a given kind, are performed. Around the clock, stationary, automated, reliable measurement and determination devices allot intended volumes of anticoagulated plasma and reagent, perform measurements and determine analyte concentrations. Control samples with assigned analyte concentration values are analysed regularly and the activities are supervised by specialized, well-trained technicians. The measurement and determination devices undergo periodic maintenance. Calibration, involving the whole procedural set up, is performed as required, particularly whenever procedural changes are made, e.g. when a new lot of reagent is introduced. Because of all this, a high level of assay reliability is reached at larger laboratories. It is no easy task to attain comparable assay reliability at smaller laboratories and at near-patient assay sites. A strategy for reliability improvement includes identifying reliability-enhancing routines practiced at larger laboratories, but not at the smaller laboratories and near-patient assay sites, and finding ways to make these routines or equivalents possible to practice also at the latter sites. Periodic maintenance of measuring and determination devices is one such measure. Calibration of the whole procedural set-up, upon introduction of procedural changes, is another. Regular analysis of control samples is a third.

For reasons of tradition, and to speed reactions, laboratory methods are typically performed at 37°C. With regard to accuracy, the temperature, it itself, is typical not crucial. If advantages in design of near-patient assay methods can be gained by performing the assay at measured ambient room temperature and accommodate the measurement in the determination, this should be considered. The reason is that measuring a temperature is much less demanding that keeping it at a defined level. The demand for 37°C in near-patient methods is a likely source of imprecision and inaccuracy. At smaller laboratories and near-patient sites, analyte concentration determinations, of a given kind, are performed sporadically. Because of this, the measurement devices are not in constant operation. The

demand for 37°C requires temperature equilibration of measurement devices and reagents in immediate connection with the determination. This, apart from consuming valuable time, becomes a source of error. Since time is precious, the equilibration time will always be at a minimum, and always be somewhat insufficient. The somewhat insufficient temperature

5   equilibration time will result in imprecise temperature definition, and cause assay imprecision. The somewhat insufficient temperature equilibration will also tend to give lower temperatures than the intended and cause assay inaccuracy. Assay time, imprecision and inaccuracy are reduced if temperature equilibration were avoided. Furthermore, assigning thermostat-heating blocks to oblivion, obviously, reduces the complexity and cost of measurement

10   devices and markedly reduces their power consumption. This, in itself, may open the way to disposable or semi-disposable, light weight, portable, manufacturer calibrated and maintained assay equipment, which may increase accuracy and reliability of near-patient analyte concentration determinations, and reduce costs.

         Further description of the background of the invention is by example, the
15   determination of prothrombin time (PT).

         According to prior art, there are two methods of PT determination. One is described in Quick A. The prothrombin time in hemophilia and obstructive jaundice. Journal of Biological Chemistry 1935;109:73-74. The other is described in Owren P. Thrombotest. A new method for controlling anticoagulant therapy. Lancet 1959; ii: 754-758. Both methods

20   are based on coagulation induced by cell membrane bound tissue factor. Hence, the reagent of both methods contains thromboplastin. However, there is an important difference. Apart from various salts and excipients, a Quick PT reagent contains only thromboplastin, whereas an Owren PT reagent also contains plasma depleted of proteins that bind to $BaSO_4$. In particular, the depleted plasma is depleted of coagulation factors II, VII and X, but not

25   depleted in two other protein components necessary for coagulation, coagulation factor V and fibrinogen. The Quick PT method relies on the sample, as a source of fibrinogen and coagulation factor V, and is profoundly affect by deficiencies and abnormalities of these. The Owren PT method is thus more specific for the factors of interest. Since coagulation factors II, VII and X, but not coagulation factor V and fibrinogen, are influenced by medical

30   treatments with vitamin K antagonists, the Owren PT method is more specific to the effects of such treatments. The treatments are highly effective in preventing thrombosis and other coagulopathies and PT assays firmly established in monitoring these treatments to assure their safety and efficacy.

         Fibrinogen is crucial in PT assays. It is the substance that forms the clot. No
35   fibrinogen means no clot, no clotting time and no PT assay. If, the fibrinogen level falls below about 0.1 g/L in the mixture of sample and reagent, clot formation is severely hampered and the clotting end-point becomes dubious. Since plasma levels of fibrinogen range down to 1

g/l, plasma to reagent ratios below 1:10 are prohibited in the Quick PT method. No such limit exists for the Owren PT method, since the reagent contains fibrinogen, and the plasma to reagent ratio can be reduced much further than 1:10.

5   The Quick PT method specifies a reaction mixture composed one volume of anticoagulated plasma and two volumes of reagent. The Owren PT method specifies one volume of anticoagulated plasma and 20 volumes of reagent. The greater sample dilution of the Owren method reduces matrix effects. This makes the Owren method more accurate that the Quick method.

10   Adaptation of a laboratory PT method to needs of smaller laboratories and near-patient assay sites requires the use of blood instead of anticoagulated plasma. According to prior art, the PT analyte is found only in the plasma portion of anticoagulated blood, and not in the cell portion. According to this, depending on the anticoagulation process and the hematocrit, the PT in one volume of anticoagulated plasma is assumed to be about the same as in 1.5 volumes of blood. Thus, according to prior art, the upholding of the assay

15   conditions of the Quick PT method or of the Owren PT method requires that 1.5 volumes of blood be mixed with 2 volumes or 20 volumes of reagent, respectively.

Better specificity for vitamin K dependant coagulation factors and better accuracy are advantages of the Owren PT method, compared to the Quick. In spite of this, prior art designers of near-patient PT methods have been more influenced by the teachings

20   of Quick than of Owren. In addition, most near-patient Quick PT method designs have clearly violated the Quick PT assay conditions by mixing blood and dried PT reagent. This has considerably reduced technical problems, but at the risk of further reduced accuracy. Prior art and inventive aspects of near-patient Quick PT methods are described in US patent 6,402,704 B1 to McMorrow, US patent 6,103,196 to Yassinzadeh et al, US patent 5,302,348

25   to Cusack et al and US patent 4,849,340 to Oberhardt.

An exception, to general design trends in near-patient PT methods, is the novi quick® PT method of November AG, Erlangen, Germany. In spite of its name, the novi quick® PT method represents an attempt to adhere to the assay conditions of the Owren PT method. To solve the near-patient problem of precise volumes, the novi quick® procedure

30   includes two novel liquid handling devices disclosed in PCT/DE99/00351 and PCT/DE99/01052 to Bertling et al. One of these is a combined glass capillary and hook with which a precise volume of blood can be added to the reagent. The capillary hook is also used to mix the blood and the reagent and, by the procedure of hooking, to determination of the clotting time. The design of the novi quick® PT abides to the philosophy of close

35   adherence to accurate laboratory methods. However, in spite of inventive efforts, the requirement of precise volumes has prohibited wide spread use.

The results of a PT determination according to the methods of Quick and Owren are commonly expressed in International Normalized Ratio (INR). The INR of plasma is derived from the quotient of the clotting time divided by the normal clotting time, NCT. To obtain the INR, the quotient is raised to an exponent that is characteristic of the assay

5   procedure. The exponent, together with the NCT, is determined by calibration. The exponent is called the International Sensitivity Index (ISI). Alternatively, PT can be expressed with respect to the PT of normal plasma, herein called PT%. Equations for inter-conversion of PT% and INR; PT%=1/(0.028*INR-0.018) and INR=[(1/PT%)+0.018]/0.028, are given in Lindahl et al. INR calibration of Owren type prothrombin time based on the relationship

10 between PT% and INR utilizing normal plasma samples. Submitted to Thrombosis and Haemostasis. Similar information is found in Gogstad G. The reporting of thrombotest in international normalized ratio (INR). Farmakoterapi 1984; 40: 88-92.

Some of the difficulties encountered in attempts to harmonize the results of PT determinations in blood and PT determinations in anticoagulated plasma are caused by

15 variations in hematocrit. According to prior art, the results are harmonized by use of one or more scaling factors. This gives reasonable results when the hematocrit is in the normal range, but not when the hematocrit is in the extremes.

Hematocrit is the fraction of the blood volume that is made up of blood cells. Hematocrit can be determined by exposing a container with blood to centrifugal forces. The

20 blood cells then form a compact mass at the bottom of the container, the volume of which is measured to determine the hematocrit. Measuring and summing the volume of each individual blood cell is another way to determine hematocrit. There are also optical methods. These are based on the fact that a good majority of blood cells are red blood cells filled with the red colored protein hemoglobin, the light absorption of which can be measured by optical

25 methods to determine the hematocrit. Optical methods to determine hematocrit are convenient and deserve special attention. Background and inventive aspects of optical determination of hematocrit are given in the following publications: U.S. patent 6,064,474 to Lee et al and U.S. Pat. No 5,277,181 to Mendelson et al. The first document discloses a method for noninvasive measurement of hematocrit and hemoglobin content of blood using

30 one or more wavelengths, e.g. 815 nm and 915 nm. The wavelengths are selected to give information on hemoglobin concentration and plasma light scatter. The second document also discloses the use of two wavelengths one at approximately 500 nm and the other at approximately 800 nm. The wavelengths are chosen because, at these, the two main forms of hemoglobin, the oxygen depleted and the oxygen saturated, show about the same

35 adsorption of light.

At smaller laboratories and at near-patient assay sites there is a need of accurate wet-chemistry methods to determine an analyte concentration in anticoagulated plasma by performing analysis on the corresponding blood, i.e. there is a need to determine an analyte concentration of anticoagulated plasma without having to prepare the same - only

5   to imagine or postulate its existence and its relevant properties. At near-patient assay sites, the methods need to be practiced in a way that circumvents the requirement of precisely defined volumes of blood and reagent. For good assay reliability, the methods should be such that regular control material, typically control plasmas and control serum with known or determined analyte concentration, can be tested. In addition, the methods should be

10   practiced on calibrated analytical set-ups that are regularly checked by analysis of control samples, and measurement and determination devices used should be periodically maintained, i.e. serviced and checked. There is also a need of methods with which the above is possible. There is a need of measurement and determination devices with which such methods can be reliably practiced, and there is a need of equipment kits for the same.

15   Specifically, all the above is needed in PT determinations used to monitor anticoagulation therapy with vitamin K antagonists.

Short description of the invention

A method to determine an analyte concentration of an anticoagulated plasma

20   by performing at least two measurements on a mixture of the corresponding blood and a liquid reagent is provided. Practice of said method also allows determination of a hematocrit of said blood, if precisely defined volumes of said blood and said reagent are mixed. Said method practiced with precisely defined volumes allows tests on control plasmas and/or control serum. Practice of said invention allows determination of said analyte concentration

25   also when imprecisely defined said volumes are mixed, if the hematocrit of the blood is known.

Measurement and determination devices for performing two or more measurements on a mixture of blood and reagent are provided. The subject devices comprise the means to perform two or more measurements, a data processor, and a read

30   only memory for storing sets of data needed for computing.

Equipment kits are provided. The subject equipment kits include reagents and subject devices for practicing the subject methods. Each subject equipment kit has an identification mark and is preferably marked with an expiration date. Reagents and subject devices, included in an equipment kit, have identification marks that are related to the

35   identification mark of the subject equipment kit. Reagents and subject devices, included in a subject equipment kit, have expiration dates that are identical to the expiration date of the subject equipment kit.

Detailed description of the invention

The invention is directed to a method to determine an analyte concentration of an anticoagulated plasma by performing at least two different measurements on a mixture of
5   a blood sample corresponding to said anticoagulated plasma and of liquid reagent. The method comprises the steps of

a) mixing a volume of said blood sample with a five-fold, or more, volume of said liquid reagent ,

b) performing said at least two measurements on said mixture, at least one of
10   which correlates with the hematocrit of said blood sample and at least one of which correlates with said analyte concentration, and

c) computing the results from the measurements when the volumes in a) are precise and accurate or when the hematocrit of said blood sample in b) is known to determine said analyte concentration of said anticoagulated plasma.

15   In an embodiment of this aspect of the invention the volume of blood in said mixture is within the range of 50% to 150% of an intended volume of blood, b)   the volume of reagent in said mixture is within the range of 70% to 120% of an intended volume of reagent, and c) computing the results to determine the analyte concentration when the hematocrit of the blood sample is known.

20   In another embodiments of this aspect of the invention, said intended volume of blood in a) is in the range of 5 to 40 µL, and said intended volume of reagent is in the range 100 to 1000 µL, i.e. the measurements are not precisely and accurately made, and preferably said volume of blood in a) is in the range of 5 to 20 µL and said volume of reagent in the range of 150 to 600 µL.

25   In yet another embodiment of this aspect of the invention, said measurements in b) are performed in a tubular container with a smallest cross section dimension of at least 4 mm, preferably with a smallest cross section dimension in the range of 5 mm to 15 mm.

In a further embodiment of this aspect of the invention, said method is calibrated, or quality assured, with anticoagulated plasma, which may be a control plasma,
30   that has been subjected to an anticoagulation process by addition of an anticoagulant selected from the group consisting of sodium, potassium and lithium salts of citrate, isocitrate, EDTA, oxalate, heparin and hirudin.

In yet another embodiment of this aspect of the invention, said anticoagulated plasma, which may be a control plasma, control serum or other control material, is a fluid
35   derived from blood, or cell, yeast or bacterial cultures, which is selected from the group consisting of blood derived fluids composed of serum, heparinized plasma, hirudinized

plasma, oxalated plasma, citrated plasma, isocitrated plasma, EDTA-plasma, heat-treated plasma and cultures fluids of cells, yeast or bacteria

In still an another embodiment of this aspect of the invention, said determination of analyte concentration is calibrated with anticoagulated blood, with known analyte concentration in the anticoagulated plasma, that has been subjected to an anticoagulation process by addition of an anticoagulant selected from the group consisting of sodium, potassium and lithium salts of citrate, isocitrate, EDTA, oxalate, heparin and hirudin.

In a still further embodiment of this aspect of the invention said analyte is selected from the group consisting of prothrombin time (PT), fibrinogen, fibrinogen degradation products, fibrin degradation products (D-dimer), activated partial prothrombin time (APTT), activated clotting time (ACT), C-reactive protein (CRP), cholesterol, and glucose.

In a yet further embodiment of this aspect of the invention said measurement that correlates with said hematocrit in b) is based on one or more measurements of light with wavelengths in the range of 800 nm to 1100 nm.

In yet another embodiment of this aspect of the invention said two or more measurements in b) are performed at ambient temperature in the range of 18° C to 40°C.

In still another embodiment of this aspect of the invention said reagent in a) contains 0.1 g/L, or more, fibrinogen.

In still another embodiment of this aspect of the invention said analyte concentration is PT expressed in INR, wherein, prior to said determination of analyte concentration in anticoagulated plasma, the analyte concentration is re-expressed in PT%.

In a further embodiment of this aspect of the invention the clotting time of said mixture in a) is one of the at least one measurement that correlates with said analyte concentration in b).

It should be understood that the blood sample used in the method of the invention may be, in addition to native blood, a manipulation of native blood in that it contains additives, such as anticoagulants, bacteriocides and drugs, or has been depleted of blood components other than the hemoglobin of the red blood cells.

Another aspect of the invention is directed to a measurement and determination device for performing measurements on blood, anticoagulated blood and/or anticoagulated plasma samples, comprising, preferably in a housing,

a) a holder for receiving a container containing liquid reagent from a specific lot, which container receives on operation of the device one of said samples for admixture with said liquid reagent,

b) an energy source,

c) a data processor,

d) a read only memory comprising a computing data set for one or more of said blood, anticoagulated blood and/or anticoagulated plasma sample admixtures, each computing data set being adapted to said specific lot,

e) measurement means for performing two or more measurements on each admixture,

f) a display that shows user instructions and computed results based on data from d) and e), and

g) control means for user control of the device, preferably a button.

Yet another aspect of the invention is directed to an equipment kit equipped with an identification mark comprising a measurement and determination device for performing measurements on blood, anticoagulated blood and/or anticoagulated plasma samples, and one or several liquid reagent(s) in container(s) equipped each with an identification mark related to said identification mark of said equipment kit.

Short description of the drawings

Fig. 1 is a diagram that shows determination of hematocrit wherein blood hematocrit (HCT) is plotted against the light absorption. The filled squares are from addition of 10 µL of various blood with known HCT to 350 µL of PT reagent. The filled triangles are from additions of various volumes of a blood with a known HCT of 44.0% to the same.

Fig. 2 is a diagram that shows the cell volume fraction of the hypothetical analyte volume of PT wherein the cell volume fraction is plotted against error in postulated anticoagulated plasma INR (%) for two groups of anticoagulated blood samples, a low HCT group (filled triangles) and a high HCT group (open squares).

Fig. 3a is a diagram showing PT according to prior art where the determined blood INR is plotted against the known plasma INR and subjected to linear regression analysis. The set is divided into three groups; a low HCT group, a high HCT group and a median HCT group. The median HCT groups were used as calibrators.

Fig. 3b is a diagram that shows PT according to the invention where the determined PT by INR in postulated anticoagulated plasma is determined and plotted against the known PT by INR of anticoagulated plasma. The clotting time values and the subgroups are as in Figure 3a.

Fig. 4 is a schematic drawing of an embodiment of a measurement and determination device according to the invention. 1A is a side view of a container inserted at an angle into the container holder of the device, 1B is a top view of the container, with an opening for adding blood, mixing blood and reagent, and hooking to detect clotting. 2 is a cross section of the beam of light from the 940 nm LED, 3 is a cavity in the container holder, said cavity harbors a thermistor for measuring the ambient room temperature, 4 is a block of wood with holes drilled to form the container holder and the light paths, 5 is a button of an

electric switch. Inside the instrument are a 9V battery, electronic circuits and a programmable integrated computer, a PIC.

Figs. 5a and 5b show pictures of an embodiment of a measurement and determination device according to the invention.

5   **Detailed description of the drawings**

Fig.1. Determination of hematocrit. Blood hematocrit (HCT) is plotted against the light absorption, i.e. the quotient of light transmitted through only the reagent (Io) and through a mixture of blood and reagent (I). The data is from Table 1. The filled squares are from addition of 10 μL of various blood samples with known HCT to 350 μL of PT reagent.

10   The filled triangles are from additions of various volumes of a blood with a known HCT of 44.0% to the same.

Fig. 2. To establish the plasma volume that contains as much of PT activity as the cell volume fraction of the blood, the b in Equation 9, to allow determination of the hypothetical analyte volume of PT, the difference between the INR of anticoagulated

15   plasma, determined according to the subject methods by use of various values for b, and the known INR, of the anticoagulated plasma, determined by an accurate laboratory method, is plotted against b. Displayed are the results of two groups of anticoagulated blood samples, a low HCT group (filled triangles) and a high HCT group (open squares). The difference is viewed as an error in the determination according to subject methods, due to imperfections in

20   the computing. At a b value of about 0.29, the error for both groups is at a minimum. The hypothetical analyte volume in anticoagulated blood is established as the sum of the plasma volume and 29% of the cell volume.

Fig. 3a. The PT, expressed inIRN, of anticoagulated plasma, was determined, according to prior art, by the clotting time of mixtures of anticoagulated blood and PT

25   reagent. A set of 40 samples of anticoagulated blood, all with known INR of their anticoagulated plasma was considered. The HCT of the samples was also known which allowed the set to be divided into three groups; a low HCT group, a high HCT group and a median HCT group. The samples of the median HCT group were used in calibration. The determined INR is plotted against the known INR and subjected to linear regression analysis.

30   The low HCT group shows a low blood INR (filled squares), the high HCT group shows high blood INR (filled triangles), and the median INR group shows intermediate blood INR (open circles).

Fig. 3b. The PT of anticoagulated plasma was also determined by subject methods, i.e. according to the invention. The same set of 40 samples of anticoagulated blood

35   samples with known anticoagulated plasma INR were analyzed. Two measurements, clotting time and light absorbance, were made on each mixture of blood and PT reagent. The clotting time values and the subgroups are as in Figure 3a. The INR values, according to subject

methods, are plotted against the known INR values of anticoagulated plasmas. For all three groups, the determined INR values are now about the same as the known INR values which demonstrates the usefulness of subject methods.

Fig. 4. A schematic drawing of the currently favored embodiment of a measurement and determination device, according to the invention, is shown. The drawing features; 1A) a side view of the container, a 10 mm in diameter polystyrene tube, inserted at an angle into the container holder of the device, 1B) a top view of the container, with an opening for adding blood, mixing blood and reagent, and hooking to detect clotting. 2) a cross section of the beam of light from the 940 nm LED, said beam passes through the container walls and its contents to reach a photodiode detector, 3) a cavity in the container holder, surrounded with model dough that extends to the exterior, said cavity harbors a thermistor for measuring the ambient room temperature, 4) a block of wood with holes drilled to form the container holder and the light paths, 5) a button of an electric switch which the operator interacts with the device, i.e. initiates light measurements, start and stop the timer, and initiates calculations and accesses determined values. Inside the instrument are a 9V battery, electronic circuits and a programmable integrated computer, a PIC. The PIC interacts with the liquid crystal display (LCD) to inter-phase with the operator. The PIC measures time, makes analog to digital conversions of measured light intensities and of temperature. The PIC also makes all necessary computations calculations to determine the PT of blood in postulated anticoagulated blood according to the invention.

Figs. 5a and 5b show pictures of an embodiment of a measurement and determination device according to the invention. Fig 5a shows addition of sample to the reagent in the container in the container holder. Fig 5b shows the result of the determination on the display. In this case the sample was anticoagulated plasma. The device has automatically noted that this was the case, should the sample have been blood, this would have been indicated. The differentiation of the samples is based on the results of the measurements on the mixture that correlates with the hematocrit.

When a range of values is provided, it should be understood that each of the intervening value, to the tenth of the unit of the lower limit, unless the context clearly indicates otherwise, between the upper and the lower limit of that range and any other stated or intervening value in the stated range, is within the invention.

It must be noted that as used herein and in the appended claims, the singular forms "a", "and" and "the" include plural referents unless the context clearly dictates otherwise. Thus, for example, reference to "a reagent" includes plurality of such reagents and reference "the device" includes references to one or more devices and equivalents thereof known to those skilled in the arts, and so forth.

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although any methods or materials similar or equivalent to those described herein can also be used in practicing or testing the present invention, the preferred

5   methods and materials are now described. All publications mentioned herein are incorporated herein by reference to disclose and describe the methods and/or materials in connection with which the publications are cited.

The publications discussed herein are provided solely for their disclosure prior to the filing date of the present application. Nothing herein is to be construed as an

10   admission that the present invention is not entitled to antedate such publication by virtue of prior invention. Further, the dates of publication provided may be different from the actual publication dates which may need to be independently confirmed.

In further describing the subject invention, the subject methods are described first. Next, a description of the subject devices is provided, followed by a description of

15   subject kits, which include the subject devices.

Methods

A way to view the method of the invention is as follows. An analyte concentration in plasma is determined by performing two or more different measurements on

20   a mixture of the corresponding blood and liquid reagent. At least one of the measurements must correlate to the hematocrit of the blood and at least one measurement must correlate to the analyte concentration of the blood. The analyte concentration of anticoagulated plasma is determined by computation, i.e. the measurement values are combined mathematically in such a way that they give the results that would have been obtained if anticoagulated plasma

25   had been prepared from the blood and this anticoagulated plasma had been subjected to analyte concentration determination by either the reference method or an accurate laboratory method. Much of the following is a disclosure of how the measurements, the measurement values, are to be combined to give the desired result, the analyte concentration of the anticoagulated plasma.

30   A measurement correlates to the hematocrit of the blood if the measured value changes with changes in the hematocrit. Two blood samples with the different hematocrits will tend to show a difference in the value of at least one of the different measurements, and the same for two blood samples with different analyte concentration. The required correlations need not be linear, nor need they be perfect. Strong linear correlations will

35   facilitate finding a mathematical combination of the measurement values to give a good determination or estimate of the analyte concentration of the anticoagulated blood, but non-linear and less strong correlations may also allow a useful practice of the invention, and is

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certainly within the scope of the invention. A corollary to the above is, that any blood analyte concentration or level that correlates to hematocrit, e.g. hemoglobin concentration or cell membrane concentration, may be interchanged for hematocrit in practice of subject methods without departing from the sprit of the invention.

5   Subject methods are practiced either at smaller laboratories or at near-patient sites. Depending on the milieu in which the subject methods are practiced, the procedures for favored practice of the invention will vary, as the prerequisites of practice of subject methods differ. Therefore, in the description of subject methods, whenever so is relevant, it will be pointed out if the practice is at a smaller laboratory or at a near-patient site. Below,

10   information on the effects of the anticoagulation process on analyte concentration determination and on the blood, relevant for practice of subject methods, is first stated. Relevant information on the behavior of the analyte in anticoagulated blood is next. This is followed by a general description of practice of subject methods at smaller laboratories and at near-patient sites, and further detailed description of the subject methods.

15   To determine an analyte concentration in anticoagulated plasma, by performing measurements on a mixture of the corresponding blood, information on the effects of the anticoagulation process on the blood is useful, as is information on the behavior of the analyte in anticoagulated blood.

          The effects of relevant amounts of anticoagulat on the measurements on the

20   measurements on the mixture of blood and reagent should be negligible. Measurements on the mixture of blood and reagent should give the same results as measurements on a mixture of blood and reagent to which reagent the relevant amount of anticoagulant has been added, all else being the same. This is not an absolute requirement for practice of the invention, but it allows preferred practice of the invention in which the method is calibrated

25   and quality assured with anticoagulated blood samples, anticoagulated plasma samples or the likes, with analyte concentrations of these biological fluids know by the reference method or an accurate laboratory method. Insensitivity to relevant amounts of anticoagulants also allows anticoagulated plasma samples to be used as controls, regular analysis of which is important to ascertain reliable performance of the practice of the subject methods.

30   In practice of subject methods, the analyte may be viewed as being distributed between the cell volume and the plasma volume of anticoagulated blood. The concept hypothetical analyte volume of anticoagulated blood describes this distribution. The hypothetical analyte volume, Vh, is an imagined or postulated volume of the blood or anticoagulated blood that contains all the analyte and has the same analyte concentration as

35   the anticoagulated plasma. The Vh is typically larger than the plasma volume of the blood or anticoagulated blood because the cell volume of anticoagulated blood has an analyte concentration greater than zero. In practice of the subject methods, the Vh is determined

from properties of the blood that are either known in advance or determined by practice of the subject methods. These properties of blood are volume and hematocrit, and possibly analyte concentration. The relationship between Vh and known or determined properties of blood is determined separately and is known prior to the practice of the invention. With

5   regard to the analyte concentration PT, used as an example describing the invention, Vh is established as the sum of the plasma volume and 29% of the cell volume of the anticoagulated blood, see Example 2. In practicing subject methods, the plasma volume and the cell volume of anticoagulated are determined by the blood volume and the blood hematocrit.

10   In smaller laboratory practice of the subject methods, the intended volume of blood and the intended volume of reagent are allotted with good precision. The blood volume is the intended blood volume and reagent volume is the intended reagent volume. Therefore, the determined blood hematocrit value and blood analyte concentration value are the true values. The analyte concentration in anticoagulated plasma may be determined from the

15   analyte concentration in blood and the Vh of the anticoagulated blood, as detailed below.

            In near-patient practice of the subject methods, the intended volume of blood and the intended volume of reagent are not allotted with good precision. The blood volume is not the intended blood volume and the reagent volume is not the intended reagent volume. Hence, the determined hematocrit value and analyte concentration value are not the true

20   values; therefore they are referred to as apparent values. To convert the unknown blood volume and apparent analyte concentration to their true counterparts, the hematocrit of the blood must be known. Knowledge of the true hematocrit of the blood is a prerequisite for near-patient practice of the subject methods. The known and the apparent hematocrit values are used to determine the true blood volume. The true blood volume and true hematocrit are

25   used to determine the Vh. The analyte concentration of postulated anticoagulated plasma is determined by use of the Vh and the determined (apparent) analyte concentration. Alternatively, the true analyte concentration is determined, and the analyte concentration in postulated anticoagulant plasma is determined by use of this value, the true (known) hematocrit and the intended blood volume.

30   The word 'intended', in the context intended blood volume and intended reagent volume, means that there is an ideal volume of blood and an ideal volume of reagent, according to the test protocol, that are to be mixed with each other. In smaller laboratory milieus, the intended volumes are accomplished with sufficient precision. In near-patient milieus, the volume of blood and the volume of reagent are perhaps within the range of 50%

35   to 150% of the intended values. The range is not necessarily the same for both volumes nor is the range necessarily symmetrical around the intended values. Since the composition of the mixture is always, more or less, ill defined, the hematocrit and analyte concentrations

determined by analysis of the mixture, can always be referred to as apparent hematocrit of the blood and apparent analyte concentration of the blood, respectively. The question the practitioner of subject methods must answer is, have the intended volumes of blood and reagent been accomplished with sufficient precision, or not. If the answer is no, then the

5   hematocrit value of the blood is required for determination of the analyte concentration according to subject methods. In the present description, smaller laboratory practice of the subject methods assumes that the intended volumes are accomplished and near-patient practice assumes they are not.

In practice of the subject methods, variations in the composition of the mixture

10   of blood and reagent are viewed as variations due to variations in the blood volume. The reagent volume is assumed to be the intended. This assumption is important in the determination of the true blood volume in near-patient practice of the subject methods. The basis for the assumption is that only the blood concentration in the mixture of blood and reagent has an impact on analyte concentration determination. This is true by two lines of

15   reasoning. One is that the reagent concentration is relatively constant at the assay conditions used. The other is that reagents are designed so that their concentrations in a reaction mixtures have little or no impact on assay response and, hence, on the determined analyte concentrations. According to the invention, the reagent volume is five-fold, or more, larger than the blood volume. At the limit, the reagent concentration in the reaction mixture is

20   5/(5+1) or 0.83 (83%). If the reagent volume is reduced to 50% of the intended volume, the reagent concentration becomes 2.5/(2.5+1) or 0.71 (71%). Thus, at the five-fold limit, a 50% decrease in the reagent volume results in only a 14% decrease in reagent concentration. At higher ratios of intended reagent volumes to intended blood volumes, the effects are even smaller. In addition, reagents have an excess of active substances and the reactions in

25   reaction mixtures are hardly influenced by reagent concentrations. A deviation in the reagent volume from the intended volume will impact on the reaction mixture in three ways. It will change the reagent concentration of the mixture, change the total volume of the mixture, and change the blood concentration of the mixture. Two of these changes are without importance; the change in reagent concentration and the change in total volume. The only

30   change that is of importance is the change in blood concentration. Variations in total volume, of the mixture of blood and reagent, deserve a little more attention. In theory, the total volume does not influence an analyte concentration determination. A small volume and a large volume have the same analyte concentration provided the composition is the same. In practice, however, there are limits. At very large volumes, the container will over-flow. At very

35   small volumes the measurements cannot be performed. The limits, within which the total volume may vary, without affecting the determinations, must be established for each individual method of the subject methods.

The two or more measurements that are performed on the mixture of blood and liquid reagent can be of any kind encompassed by prior art. The measurements can be electromagnetic, electric, magnetic, rheologic, calorimetric, stoichiometric or temporal. The electromagnetic measurements include measurements of all sorts of electromagnetic

5   radiation; visible, ultraviolet, infrared light, microwaves, radiowaves etcetera. Electric measurements include measurements of all sorts of electrical phenomenon such as resistance, impedance, potential, current and capacity. Caloric measurements include temperature and related analytes. Stoichiometric measures include all sorts of counting; cell counting, and radionuclide disintegration counting etcetera. In preferred practice, one

10   measure is selected for each analyte, but this is in no way necessary. Two optic measurements, e.g. measurements at two wavelengths, may be linearly combined to obtain two analyte concentration determinations. Alternatively, two or more optical measurements at the same wavelength, but separated in time, can be used to determine two analyte concentrations. The time between two occurrences in the mixture is such a measure. Two or

15   more measurements are needed to determine two analyte concentrations and three or more are needed to determine three analyte concentrations and so forth. In a current practice of subject methods, one optic measurement and time measurement, based on rheological occurrences, are performed on the mixture. The optic measurement is used to determine hematocrit and the rheologic measurement to determine PT. With the rheological

20   measurement a clotting time is determined. A clotting time could be used to determine any coagulative analyte concentration such as activated partial prothrombin time (APTT) or activated coagulation time (ACT).

In the subject methods, the phrase 'analyte concentration' pertains to any property of matter that is related to the number of copies of some observable or imaginary

25   entity per unit of volume. Analyte concentration is thus stoichiometric in nature. Analyte concentration determination in blood is related to determining the number copies of such entities per unit volume of blood. If the blood is diluted the analyte concentration falls. This does not necessarily apply to the analyte concentration by a given expression. Analyte concentration by a given expression is not necessarily proportional to a concentration of

30   some observable or imaginary entity. An example is acidity. Acidity is an analyte concentration related to the number of imagined H^+ ions per unit volume. Acidity is commonly expressed in pH. Acidity by pH is clearly not proportional to the concentration of H^+ ions. An analyte concentration by a non-proportional expression can be re-expressed to become proportional. For example, acidity by pH can be re-expressed as acidity by 10 to the power of

35   –pH, to perhaps become proportional. Another example of analyte concentration is prothrombin time (PT). This analyte is related to the concentration of coagulation factors, particularly coagulation factors II, VII and X. Determination of PT can, hence, be viewed as

determination of an analyte concentration. Commonly used expressions of PT are clotting time and INR. Expressed by clotting time or by INR, the PT concentration is not proportional to the concentrations of clotting factors. For practice of subject methods, it is of importance that hematocrit is by proportional expression; other analyte concentrations determined in

5 practice of the subject methods may be expressed by any expression, proportional or not. Certain determination procedures, particularly the computations, disclosed herein require that the analyte concentration be by proportional expression. To ascertain that an analyte concentration is by proportional expression is should be checked that the determined apparent analyte concentration is proportional to the blood concentration in the reaction

10 mixture. The experimental data in Table 1 allows such a check on hematocrit.

In near-patient practice of the invention, analyte concentration by proportional expression allows straightforward determination of analyte concentration in blood. If the apparent and true analyte concentrations in blood are At and Aa, respectively, and the apparent and true (known) hematocrit are HCTa and HCTt, respectively. The following

15 applies:

$$At = Aa*HCTt/HCTa \qquad\qquad \text{Equation 1}$$

The true analyte concentration in blood and the true hematocrit are sufficient to determine an analyte concentration in anticoagulated blood, since the blood volume then can

20 be assumed to be the intended.

If the analyte concentration is not by proportional expression, the calculation may proceed by determining the true blood volume according to the following equation:

$$Vbt = Vbi*K*R/(R-K+1) \qquad\qquad \text{Equation 2}$$

25

The true and intended blood volumes are Vbt and Vbi, respectively. K is the ratio of HCTa to HCTt, and R is the ratio of the intended reagent volume, Vri, to the intended blood volume, Vbi.

To check if an analyte concentration is by proportional expression, the blood

30 concentration in the mixture of blood and reagent is needed. This, and other concentrations in the mixture, can be determined with the following equation:

$$X = (Q+Q*R)/(Q+R) \qquad\qquad \text{Equation 3}$$

35 In Equation 3, R is Vri/Vbi, as in Equation 2, and Q is Vb/Vbi. Equation 3 gives normalized concentration values, i.e. concentration values that are unity (100%) when Q is one. Equation 3 informs that X is equal to Q when R goes towards infinity. Equation 3 is

handy in calibration of hematocrit by allowing the use of various volumes of a few calibrator blood samples to cover a wide range of hematocrit values, see Example 1. In currently favored practice of subject methods, R is 35. At this condition the difference between X and Q is pronounced only at higher Q values.

5   One general way to determine an analyte concentration in postulated anticoagulated plasma, App, is by use of the concept hypothetical analyte volume, Vh, detailed below. The analyte concentration in blood is determined with respect to the analyte concentration in anticoagulated blood expressed as analyte concentration in anticoagulated plasma. To do this, the analyte concentration determination is calibrated using appropriate

10   volumes of anticoagulated blood calibrators with known analyte concentrations in their anticoagulated plasmas. These calibrators have a known mean hypothetical analyte volume, Vhm. The appropriate volume of calibrator is the intended volume of blood after subjection to the postulated anticoagulation process. The dependence of the determined analyte concentration on the Vh is established as the differential dA/dVh. The analyte concentration

15   in blood, Ab, and its associated Vh is determined. The desired analyte concentration in postulated anticoagulated plasma, App, is obtained by:

$$App=Ab+\int(dA/dVh)^*dVh \qquad\qquad\qquad Equation\ 4$$

20   The integration is from Vh to Vhm. In Example 3, App is determined according to Equation 4. In the example, the differential is approximate by $\Delta A/\Delta Vh$, i.e. by macroscopic change in A, (A2-A1) divided by the macroscopic change in Vh, (Vh2-Vh1).

An analyte concentration is, to some degree, dependant on the method used in its determination. Because of this, a characteristic property of the method is often indicated.

25   An example is the analyte concentration hematocrit. Hematocrit may be determined by measuring the volume of blood cells, or by measuring light. Depending on the method used, the analyte concentration may be referred to as volumetric hematocrit or photometric hematocrit, respectively. If nothing is said about the method used, the interpretation can be either broad or narrow. A narrow interpretation is that a reference method has been used. A

30   broad interpretation is that any known method has been used. In the subject methods, the phrase 'an analyte concentration' should be interpreted in the broadest, most non-limiting way. In the context of the subject methods, the phrase 'analyte concentration in postulated anticoagulated plasma' refers to the analyte concentration that is obtained, by any method, if the blood is subjected to a postulated anticoagulation process and the analyte concentration

35   is determined in the anticoagulated plasma. The word postulated in the above simply indicates that the substance has not been at hand, not been prepared, only imagined. In many places in this text it has been omitted. It is added for clarity, but is perhaps defeating it

purpose. In preferred practice of checking the accuracy and precision of the subject methods the analyte concentration in the anticoagulated plasma is determined by an accurate laboratory method. The analyte concentration in postulated anticoagulated plasma, determined by practice of the subject methods, is not necessarily identical to this value. The spirit or gist of the subject methods is, that the value obtained by determination of analyte concentration in anticoagulated plasma, by practice of the subjects methods, is close to the value that would have been obtained if determination had actually been performed on the actual anticoagulated plasma.

In the subject methods, hematocrit is determined by any method known to capable of determining hematocrit. In preferred practice the hematocrit is determined by measurement of transmitted light with wavelengths in the range of 800 nm to 1100 nm. The hematocrit determination is calibrated with blood samples with known hematocrit values. The hematocrit values are known by an accurate laboratory method. As pointed out above, to practice the invention, the measurements need not be translated into hematocrit, they may be translated into some other analyte concentration or level that correlates to hematocrit, or they may not need to be translated at all.

In the subject methods, the phrase 'the effects on blood volume and hematocrit of (the postulated) anticoagulation process' pertains to typical, or average effects of the process.

Three types of anticoagulation processes are commonly used in clinical diagnostic method, anticoagulation with EDTA, heparin or citrate. Two of these processes, anticoagulation with EDTA and anticoagulation with heparin, have only minute effects on blood volume and hematocrit. The citrate anticoagulation process, as commonly practiced, has pronounced effects. The typical citrate anticoagulation process consists of adding one volume of 0.11 M or 0.13 M tri-sodium citrate to nine volumes of blood. This affects the total blood volume and the hematocrit. The citrate solution is hypertone and shrinkage of blood cells is expected, and may need to be taken into account. If citrate anticoagulation applied to blood with a volume Vb and a hematocrit HCT, the volume of anticoagulated blood and its hematocrit, Vbcit and HCTcit, respectively, are given by the following:

$$Vbcit = Vb*10/9 \approx 1.111*Vb \qquad\qquad \text{Equation 5}$$

$$HCTcit = HCT*9/10 \approx HCT/1.111 \qquad\qquad \text{Equation 6}$$

The plasma volume and the cell volume of the postulated anticoagulated blood, Vpcit and Vccit, respectively, are given by:

$$Vpcit=Vb^*(1.111-HCT) \qquad\qquad \text{Equation 7}$$

$$Vccit=Vb^*HCT \qquad\qquad \text{Equation 8}$$

5   If x% shrinkage of the blood cells is known to occur, the hematocrit decreases by x% and the volume of the plasma increases by the volume the cells have shrunk.

In preferred practice of the invention, the determination of analyte concentration in blood is calibrated with the appropriate volume of anticoagulated blood calibrators with known analyte concentrations in their anticoagulated plasma. In the calibration procedure, a

10   hematocrit value is obtained for the corresponding blood of each calibrator from which the hematocrit of the calibrators can be determined. This hematocrit allows determination of the hypothetical analyte volume of each calibrator, as detailed below. For example, if a subject method is to be performed with an intended blood volume of 10 µL and an intended reagent volume of 350 µL and the postulated anticoagulation process is citrate anticoagulation, the

15   method is calibrated by use of 11.11 µL of citrate anticoagulated blood, according to Equation 4. An apparent hematocrit of the calibrator is obtained. Since 11.11 µL has been used instead of the intended 10 µL, the hematocrit of the calibrator is very nearly the apparent hematocrit divided by 1.111. For best accuracy the apparent hematocrit should be divided by the normalized concentration of Equation 3. Inserting a Q of 1.111 and R of 35

20   results in a normalized concentration, the X value, of 1.108.

Determination of the hypothetical analyte volume requires information that may be obtained by separate experiments. The hypothetical analyte volume, Vh, of anticoagulated blood is a volume that contains all the analyte and has the same concentration as the anticoagulated plasma. In preferred practice of the invention, the model

25   for Vh is the sum of the plasma volume and a fraction of the blood cell volume of anticoagulated blood. If the fraction is b and the volume of anticoagulated blood is Vab, hematicrit of anticoagulated blood is HCTab, Vh is given by:

$$Vh=Vab^*(1-HCTab+b^*HCTab) \qquad\qquad \text{Equation 9}$$

30

For citrate anticoagulation, Vab and HCTab are obtained from the blood volume, Vb, and the blood hematocrit, according to Equations 5 and 6. The fraction b is established in separate experiments. Example 2 describes such experiments with citrate anticoagulated blood and the analyte concentration PT. In this case b was found to be 0.29.

35   If the determination of the analyte concentration in blood has been calibrated with the appropriate volumes of anticoagulated blood calibrators with known analyte concentrations in anticoagulated plasma, as described above, the determination of analyte

concentration in blood, Ab, equals the analyte concentration in postulated anticoagulated blood provided the Vh is equal to the mean Vh of the calibrators, Vhm. If Vh differs from Vhm, and the analyte concentration is by proportional expression, the analyte concentration in postulated anticoagulated plasma, App, is determined by the following expression;

5

$$App=Ab*Vhm/Vh \qquad\qquad\qquad Equation\ 10$$

The desired result, App, is thus conveniently obtained if the analyte concentration is by proportional expression and the concept of hypothetical analyte volume has been filled with meaning. If the analyte concentration is not by proportional expression it may be re-expressed to become proportional. Equation 9 can then be applied after which, if so is desired, the analyte concentration can again be re-expressed into the original expression. The above described procedure to obtain the analyte concentration in postulated anticoagulated blood is just an example of one of many possible procedures by which the analyte concentration in postulated anticoagulated plasma may be obtained, by practice of subject methods of the invention. The practice of the subject methods results in determination of blood hematocrit and blood analyte concentration by some expression. It is obvious from the above that the mode of expression is dependent on the calibration procedure and the calibrators. Whatever the expression, practice of the subject methods of the invention provides a way by which an analyte concentration in anticoagulated plasma may be determined, without having to prepare the anticoagulated plasma.

It is important to note, that a proportional expression can always be obtained over some analyte concentration range. The analyte concentration of blood, or better anticoagulated blood with known analyte concentration in plasma, is always available in some expression or a procedure to determine the analyte concentration in anticoagulated blood is meaningless. By varying the volume of anticoagulated blood and plotting the anticoagulated blood concentration, the X of Equation 3, against the analyte concentration expression, the anticoagulated blood concentration can be expressed as a function of the analyte concentration by the given expression. In the analyte concentration range which includes the anticoagulated blood concentration 1 and where the anticoagulated blood concentration either rises or falls continuously with the analyte concentration, the function of the analyte concentration is by definition a proportional expression of the analyte concentration.

In smaller laboratory practice of subject methods, the blood volume is known, because it is the intended blood volume. The determined apparent hematocrit and apparent analyte concentration are the true hematocrit and true analyte concentration, of the blood. By these true values of the blood, the analyte concentration in anticoagulated blood is

determined. In preferred practice of the subject methods, the analyte concentration determination is calibrated with anticoagulated blood calibrators with known analyte concentration in their anticoagulated plasma, as determined by an accurate laboratory method. The hematocrits of these calibrators are determined in connection with the

5   calibration. The mean hematocrit of the calibrators and the hematocrit of the blood are thus known. By use of the determined analyte concentration of the blood, the hemaocrit of the blood and the mean hematocrit of the calibrators, the desired analyte concentration in postulated anticoagulated blood is determined. The concept hypothetical analyte volume is useful in the determination. There are innumerous procedural alterations by which the

10   desired result may be obtained from the blood hematocrit and the blood analyte concentration. To give an idea of the possibilities, examples are provided. Otherwise, the information required to convert known analyte concentration and hematocrit of blood into analyte concentration in postulated anticoagulated plasma can be in many forms, e.g. in the form of tables or in the form of two, or more, variable functions.

15   In near-patient practice of the subject methods, the determination of analyte concentration in (postulated) anticoagulated blood is performed in a similar as in smaller laboratory practice. The difference resides in the fact that intended volumes of blood and reagent cannot be accomplished. Because of this, the composition of the mixture of blood and reagent is ill defined. To compensate for this imperfection, the hematocrit of the blood

20   must be known. With the known, or true, hematocrit, HCTt, and the determined apparent hematocrit, HCTa, the true blood volume is determined by use of Equation 2. With the true blood volume and the true hematocrit value and the corresponding (apparent) analyte concentration, the analyte concentration in postulated anticoagulated plasma is determined. A preferred way to perform the determination is by use of the concept hypothetical analyte

25   concentration as described above. As pointed out above, there are numerous ways to perform the operation. Equation 3 presents one possibility; tables and multi variable functions are other. If the analyte concentration is by proportional expression, the determination of analyte concentration in postulated anticoagulated plasma is straightforward. Equation 3 gives the true analyte concentration. The true analyte concentration value, the true (the

30   intended) blood volume and true (known) hematocrit are used to determine the desired analyte concentration of postulated anticoagulated blood, by use of Equation 9 and the concept hypothetical analyte volume. In near-patient practice of the subject methods of the invention, determination of apparent hematocrit and apparent analyte concentration by two or more measurement on the same mixture of blood and reagent is crucial. The common

35   mixture links the apparent hematocrit value and the apparent analyte concentration value, which makes the desired determination possible. Apart from being necessary, the determinations are convenient to perform.

There are many medical diagnostic analyte concentrations suited for determination by the subject methods. These analytes concentrations include, but are not restricted to, analyte concentrations of a group of analyte concentrations comprising prothrombin time (PT), fibrinogen, fibrinogen degradation products, D-dimer, activated partial

5   prothrombin time (APTT), C-reactive protein (CRP), cholesterol, and glucose.

The anticoagulated plasma is to be interpreted in a broad sense. It includes all types of non-coagulating fluids obtained from blood, which are used as samples for determination of analyte concentrations. Said fluids are contained in the following group of fluids, but are not restricted to membership in that group. The group consists of serum,

10   heparinized plasma, hirudinized plasma, oxalated plasma, citrated plasma, isocitrated plasma, EDTA-plasma and heat-treated plasma.

In practice of the subject methods, the (postulated) anticoagulation process, to which the blood may be subjected, includes addition of an anticoagulant selected from the group of anticoagulants composed of sodium, potassium and lithium salts of citrate,

15   isocitrate, EDTA, oxalate, heparin and hirudin.

It is construed within the subject methods that the two or more measurements performed on a mixture of blood and reagent are performed at ambient temperature in the range of 18° C to 40°C. To accomplish this, calibration is performed at several temperatures within the mentioned range and the calibration parameters are established as functions of

20   temperature.

In smaller laboratory practice of the subject methods, apart from the desired analyte concentration in postulated anticoagulated blood, the hematocrit of the blood is obtained. This hematocrit value may be used to increase the reliability of the analyte concentration determination. The value may be compared with reference values or with

25   previously determined values. If the hematocrit value is unreasonable, this may be used as a criterion to disqualify the analyte concentration determination.

At the near-patient site, most of the information contained in the known hematocrit value is consumed in defining the volume of blood mixed with the intended volume of reagent and/or the true analyte concentration. Yet, some limits may be established

30   on the maximal difference between HCTa and HCTt. Such limits may be used to increase the reliability of the analyte concentration determination. A differences exceed the limit could disqualify the analyte concentration determination. Also, repeated determinations over time, can give indications that the hematocrit of the patient has drifted, and in need of a renewed determination.

35   PT is an analyte concentration that can be determined in the postulated anticoagulated plasma by practice of subject methods. PT, expressed in INR, is determined by one or more measurement on a mixture of blood and reagent. The determination may be

facilitated by re-expressing PT by the non-proportional expression INR, into PT by the proportional expression PT%. This can be done with the equation PT%=1/(INR*0.028-0.018). The PT% in postulated anticoagulated plasma is then determined by some chosen procedure. Prior to reporting the PT in postulated anticoagulated plasma, PT by PT% may be

5   re-expressed into PT by INR. This is done by use of the inverse of the mentioned function, INR=[(1/PT%)+0.018]/0.028. The re-expression of PT is not necessary. In preferred procedures of practicing the invention, re-expression of PT is not performed. The PT in postulated anticoagulated plasma is determined by use of the PT by INR and the blood hematocrit. Such a procedure may be preferred because they involves fewer calculations.

10   The theme of the present work is to adapt well performing wet-chemistry procedures to the needs of near-patient testing. By definition, wet-chemistry determination requires that the sample is diluted in the reagent. In all practice of subject methods, the sample is blood and the minimal dilution is five fold. To determine hematocrit by light transmission, dilution of the blood is necessary to avoid short optical path lengths. Short

15   optical path lengths means small inner dimensions of the container for the mixture of blood and reagent, which disturbs practice of the subject methods. Intended practice of subject methods involves manual steps, e.g. the contacting and mixing of blood and reagent, and the dimension of sample container must accommodate this. Inner dimensions of the container smaller than 4 mm make the manual steps impractical or even impossible to perform. In

20   preferred practice of subject methods, a tubular container with circular cross section of 8 mm is used. Acceptable cross-sections, i.e. light paths, in tubular containers are in the range of 4 to 16 mm. Also to volume of blood samples its practical limits. The intended source of blood in practicing the subject methods is a pricked fingertip and the blood is to be picked up manually and transferred to the reagent, inside the container. The blood sample must be in

25   the range of 5 μL to 40 μL. The dimensions of the container and ratio of reagent volume to blood volume limits blood volume to the range of 100 μL to 1100 μL. As will be explained below, only light within a limited wavelength span, range of 800 to 1100 nm, is acceptable. In most preferred practice, the cross-section dimensions of the tubular container is in the range of 5 mm to 15 mm, the blood volume is in the range of 5 mL to 20 mL, the reagent volume in

30   the range of 150 μL to 600 μL.

In preferred practice of subject methods, hematocrit is determined by measuring the intensity of light transmitted through a mixture of blood and reagent. In currently most preferred practice, the intensity of light transmitted through the reagent alone is also measured, and hematocrit of the blood is determined from the quotient of the

35   measured intensities of light transmitted through the reagent and of light transmitted through the mixture of blood and reagent. By this preferred practice, experimental fluctuations tend to cancel. Source of fluctuations may be the light source, the optical properties of the reagent or

the optical properties of the container. In preferred practice, the wavelength of the light is in the range of 800 nm to 1100 nm. This light is in the near infrared part of the light spectrum and can hardly be detectable by the human eye. Light in this wavelength range is preferred because it is absorbed by oxygen saturated and oxygen depleted forms of hemoglobin to

5   about equal, and relatively small, degree. Low absorption is important because it allows use of relatively long optical path lengths, e.g. 8 mm, the diameter of the transparent plastic tubes used as containers in the currently most preferred practice of the invention. At wavelengths above 1100 nm, the light absorption by water increases dramatic to make optical determination of hematocrit difficult. At wavelength below 800 nm, in the range of 600 nm to

10   800 nm, the two mentioned forms of hemoglobin absorb differently whereby a source of error is introduced. At still shorter wavelengths, light absorption by hemaoglobin is very strong. This either precludes the preferred optical path lengths of 4 mm or more, or necessitates excessive dilution of blood in reagent.

        In practice of subject methods, the mixture of an intended volume of blood and

15   an intended volume of reagent can be accomplished in many different ways. It is conceived within the scope of the invention, that the blood may first be diluted, e.g. in 9 g/L sodium chloride, and then mixed with the reagent. Alternatively, the reagent may be in several components, e.g. a first component, a second component etc. In the practice of subject methods, it is the final ratio to reagent volume to blood volume that is of importance. The

20   contacting of blood and reagent is when the last component necessary for the reactions between blood and reagent, is added.

        Prothrombin time (PT) is an analyte concentration that may be determined in postulated anticoagulated plasma by practice of the subject methods. In preferred practice the following applies. The contacting of blood and PT reagent signifies the addition of the last

25   component necessary for the coagulation reactions to commence in a mixture of blood and PT reagent. The contacting defines the start of the clotting reactions and the start of time measurement. When clotting is first detected, the time measurement is stopped and the clotting time is obtained. It is conceived that any of the methods used to determine clotting time, according to prior art, can be used to determine clotting time in practice of the

30   invention. These methods include detection of clotting by rheological, mechanical and optical means. In preferred practice, the clot is detected by hooking. Clot detection by this mean allows simple automation of clot detection. When the clot attaches to the hooking rod and is removed from where the beam of light passes through the container, the intensity of light reaching the detector increases dramatically. This surge in light intensity is readily detected

35   and can be used to automatically register the clotting time. It is preferable to calibration of a PT determination with anticoagulated blood with known plasma INR values. It is preferred, that the plasma INR values are by an accurate Owren PT laboratory method. Plasma INR

values by a Quick PT laboratory method may also be used, but a greater number of samples are then required to obtain comparable accuracy.

Devices

5   Measurement and determination devices for performing subject methods are provided. Subject devices contain, 1) a data processor, 2) a read only memory, 3) calibration data for hematocrit, said hematocrit calibration data stored in said read only memory, 4) calibration data for said analyte, said analyte calibration data stored in said read only memory, 5) means of performing two or more measurements on mixture of blood and

10   reagent.

         The data processor will perform all calculations necessary to determine the blood hematocrit, a blood analyte concentration and the analyte concentration in postulated anticoagulated plasma. The calibration data necessary to determine the blood hematocrit and a blood analyte concentration are stored in a read only memory associated with

15   processor. The read only memory is a functional read only memory in the sense, that an operator cannot alter what is stored in the read only memory. Only the manufacturer inserts data into the read only memory. One kind of data can be entered by the user, or by personnel of medical center to which the user is associated. The data concerns the blood of an individual, the identity of the individual to which he blood belongs and dates and

20   information of time and places.

         A preferred embodiment of subject devices features a container holder into which a tubular container with a mixture of blood and reagent is inserted. When in position, the longitudinal axis of the tubular container and the direction of the earth's gravitational force will form an angle in the range of 25 to 65 degrees. In preferred practice, the container is a

25   plastic tube in which clotting time can be measured by hooking. When the clot attaches to the hooking rod, it is more easily moved if the container is slanted. The clot needs only to be lifted a fraction as high against the earth's gravitational field as would have been the case if the container were perpendicular. This makes for a more reliable manual or automatic measurement of clotting time. In preferred embodiment, the tubular container is slanted 25 to

30   65 degrees compared to the direction of the earth's gravitational field.

Kits

         An equipment kit for determination of an analyte concentration in postulated anticoagulated plasma is provided. Subject kits are individual marked with an identification

35   mark, which indicates the lot to which the individual kit belongs. Subject kits contain reagents and subject devices. Each kit of subject kits contains reagents to perform subject methods. Each container of reagent in a subject kit has an identification mark that is related to

identification mark of the subject kit. Each of the subject kits contains one or more subject devices. Each subject device in one subject kit has an identification mark that is related to the identification mark on the subject kit. Each individual of the subject kits has an expiration date; the same expiration date applies to reagents and subject devices that are contained in

5   the individual subject kit. In this context, 'related identification marks' mean that the identification marks have something in common. The identification mark may be identical, or they may share a common feature that indicates that they belong together. The identification mark of the kit is its lot number, or its functional equivalent. Related identification marks could be the same lot number, or functional equivalent, as that of the kit, or a lot number, or

10   functional equivalent, that contain an identical string of digits and letters, or the like, to indicate that the identification mark of the kit and that of the reagent and that of subject device constitute a unit that should be used together. The related identification could even be totally different, and be linked only via information indicating that they are linked. This information could be manifested in a register to which the user has access or be indicated on

15   the kit or inside the kit, e.g. on the instructions of use. The related identification mark could even be purely functional. The device will only function with reagents of the same kit lot as the device.

        The kit has an expiration date. The same expiration date applies to reagents and the device that are part of that kit. When the expiation date is passed, the kit is not to be

20   used, nor the reagents and the device.

        The function of the related identification marks and the common expiration date is to assure that the device has undergone maintenance, i.e. has been checked with regard functionality, within some defined period of time. The device has undergone a quality assurance procedure at the time when the kit was released for sale by the manufacturer.

25   From that time and until the expiration date, the device may be used.

        The subject device has a read only memory in which calibration data for the determination of hematocrit and an analyte concentration using the reagents which are a part of the same subject kit as the subject device. By this mechanism, the subject device and the reagents have been calibrated together.

30   The subject kits have the property of allowing that a periodically maintained subject device is used together with reagents that have been calibrated together. This is, reliability-enhancing measures, typical of larger laboratories, are installed at near-patient assay sites.

        A use of subject kits is envisaged that involves interaction between near-patient

35   assay sites and smaller laboratories, the latter including subdivisions of larger laboratories. A first practice of subject methods, using subject devices and reagents of subject kits, on the blood, or anticoagulated blood, of a certain patient, is at a smaller laboratory. Apart from the

analyte concentration in postulated anticoagulated blood, the practice of subject methods at a smaller laboratory site also yields the hematocrit of the blood of the patient. This hematocrit, together with the patient's identity, the date and the time of day, is entered into the memory of the subject device. The subject kit is thereby ready for near-patient analysis of

5   an analyte concentration of that patient. Near-patient analysis of the blood, of the patient in question, will allow determination of analyte concentration determination that hardly affected by an inability to precisely allot volumes of blood and reagent, provided that changes in hematocrit are moderate. Typically, except at situation of heavy bleeding and blood transfusions, changes in hematocrit are slow. The hematocrit of a given individual tends to

10 be stabile over time. To check the hematocrit, and for other reasons, periodic interactions between the practitioner of the subject methods and the smaller laboratory is recommended.

Summary of the aspects of the invention

Methods for determination of an analyte concentration of anticoagulated plasma

15 by analyzing a mixture of a volume of blood and an intended volume of reagent are provided. Said postulated anticoagulated plasma is from blood subjected to a postulated anticoagulation process. Said subject methods are characterized in that; a) said intended volume of reagent is five-fold, or more, larger than said intended volume of blood, b) two or more measurements are performed on said mixture, d) hematocrit of said blood is

20 determined, e) said analyte concentration of said blood is determined.

Measurement and determination devices are provided. The measurement and determination devices will perform two or more measurements on a mixture of blood and reagent and will perform the necessary calculations to determine an analyte concentration in postulated anticoagulated plasma. Subject devices comprise; 1) a data processor, 2) a read

25 only memory, 3) calibration data for determination of hematocrit, said hematocrit calibration data is stored in said read only memory, 3) calibration data for determination of said analyte concentration, said analyte calibration data is stored in said read only memory, 4) means of performing two or more measurements on a mixture of blood and reagent.

Equipment kits for determination of an analyte concentration in postulated

30 anticoagulated plasma are provided. Each individual of a lot of subject kits has an identity mark and an expiration date characteristic of the lot to which the individual kit belongs. A subject kit comprises; 1) reagents, said reagents have an identification mark, said identification mark is related to said identification mark of said equipment kit, said reagents have an expiration date which is identical to said expiration date of said equipment kit, 2) a

35 measurement and determination device, said measurement and determination device has an identification mark, said identification mark is related to said identification mark of said

equipment kit, said measurement and determination device has an expiration date which is identical to said expiration date of said individual equipment kit,.

It is to be understood that this invention is not limited to the particular practices and embodiments described, as such may, of course, vary. It is also to be understood that
5 the terminology used herein is for the purpose of describing particular practices and embodiments only, and not intended to be limiting, since the scope of the present invention is limited only by the appended claims.

EXAMPLES

10 **Materials and methods**

A measurement and determination device, a subject device according to the invention was constructed. For a schematic drawing see Figure 4. The device has a container holder in which a container, a 10 mm outer diameter, 63 mm in length, polystyrene tube, can be obliquely inserted. When inserted, about 22 mm of the bottom end is inside the
15 container holder. Inside the container holder, about 8 mm from the bottom of the inserted container, a beam of light, from a light emitting diode (a 940 nm LED, Everlight IR204), is directed, perpendicular to the inserted container, along one of its diameters, towards a photodiode detector (a 900 nm peak sensitivity photodiode, Infineon SFH 2030F). The photodiode is connected to an operation amplifier (between pins 2 and 8 of a light to voltage
20 converter, Burr-Brown OPT 101, with its optical area blackened). The amplifier applies a voltage, proportional to the intensity of the incident light, onto a 10-bit analog-digital converter (A/D converter) input of a programmable integrated computer (Microchip PIC16F873-201/SO). A digital representation of the voltage, a measure of light, is displayed on a liquid crystal display (LCD, Seiko L167100J). The device has one button with which the operator
25 interacts with the PIC and its software. A press of the button moves the processor activities from one part of the software to another or start and stops activities. In one part of the program, the button will start and stop a timer function of the PIC, which determines the clotting time. There is circuitry for delivering a voltage proportional to the ambient temperature to another A/D converter of the PIC. In execution of relevant parts of the
30 program, the PIC is programmed to display measures of light and clotting time on the LCD. The PIC stores calibration values for determination of PT within the temperature range 18 to 40°C; NCT and ISI as functions of temperature. At the end of a determination, the hematocrit of the blood and the PT activity of anticoagulated blood are displayed. In Example 5, the measuring and determination device, according to the invention, had been redesigned and
35 fitted with improved software. The device had been given the capability to start time measurements automatically when the blood sample was added and mixed. The clotting time was detected automatically by optical means. The device performs necessary computations

automatically at the time of clot detection and displays the results of the analyte concentration determination automatically on the LCD.

In preferred practice of the invention, a container with 350 µL PT reagent was placed in the sample holder. The intensity of light (Io), transmitted through the PT reagent, is
5   measured. Blood is added to the reagent, i.e. contacted with the reagent, and mixed. At the moment of contact, time measurement is commenced. The intensity of light (I) transmitted through the mixture and reaching the detector, is again measured. At the moment of clot detection, the time measurement is stopped. In Examples 1, and 3, precisely defined volumes of blood were added to the PT reagent. This was with a pipette, adjustable in the
10   range 2 µL to 20 µL. In Example 2, the addition was with a semi-quantitative volumetric device, a platineuse. In all examples, precisely defined 350 µL of PT reagent was used. In all examples, the mixing of blood and reagent, and the hooking to detect clotting, were with the platineuse. In Example 5, 20 µL of blood, or for calibration anticoagulated plasma, was added to and mixed with 400 µL of PT reagent.

15   Platineuses of plastic with a 10 µL loop, Sevant Oy, Helsinki, Finland, were delivered under article number 007-2510 by Labora AB, Upplands Väsby, Sweden.

Owren PT reagent GHI 131 was from Global Hemostasis Institute MGR AB, Linköping, Sweden.

20   Decoded blood samples, either EDTA anticoagulated for hematocrit and hemoglobin concentration, or citrate anticoagulated samples for PT determination, were patient plasma samples remitted to the Central Laboratory of the University Hospital, Linköping, Sweden, for the determinations. The hematocrit and hemoglobin of the EDTA samples and the PT activity of the citrated samples were known by accurate laboratory
25   methods, the routine procedures of the mentioned central laboratory

The clinical experiments, experiments involving decoded patient samples, were performed in collaboration with Professor Tomas Lindahl and approved by the local ethical committee of the named university hospital.

30   **Example 1**

Six samples of EDTA anticoagulated blood with known, or true, hematocrit values, HCTt, as determined by an accurate laboratory method, were analyzed using a subject device of the invention. A blood volume (Vb) of each sample was added to and mixed with an intended volume of 350 µL of PT reagent. The intended blood volume was 10 µL.
35   Prior to, and after, the addition and mixing of blood, the intensities of light transmitted through the reagent (Io) and through the mixture (I) were measured. From one of the six samples, sample 6AS with a HCT of 44.0%, experiments with various blood volumes, 4, 6, 8, 12, 14,

16 and 18 µL, are also performed. These blood samples, called 4S through 18S,

respectively, are assigned an HCT values based on the dilution of the blood according to

Equation 3. Thus, e.g. the HCT of 4S is 44.0% multiplied by the X in Equation 3, 17,9%. The

known HCT values are plotted against I/Io and subjected to linear regression analysis. This

5   yielded the regression equation HCT=2.03*(Io/I)+9.37 with r^2=0.98. The data is presented in

Table 1 and the plot in Figure 1. The Io/I values in Table 1 are inserted into the regression

equation to generate apparent HCT values. With the HCTa value and the known, or true,

hematocrit values, HCTt, the blood volumes are determined by se of Equation 2. The

determined blood volumes, Vbd, are compared with the known volumes of added blood, Vb.

10   It is found that the determined blood volumes were 101%±7% (mean±CV) of the known

blood volumes. The same is found also in comparing HCTa and HCTt. The hemoglobin

values of the samples are also known, linear regression analysis of the hemoglobin (Hb), in

grams per liter, plotted against Io/I yielded Hb=7.17*(Io/I)+29.8 with r^2=0.99, data not shown.

The equation HCT=2.03*(Io/I)+9.37 is used to generate apparent HCT values in Example 2

15   and Example 3. The use of smaller and larger volumes of blood than the intended, and the

use of Equation 3 to determine normalized concentrations is practical. It allows access to

wide HCT range by use of only a limited number of samples.

Example 2.

20   An example of near-patient practice of a subject method is given. Two citrate

anticoagulated blood samples, Sample 1 and Sample 2, with known HCT values were

subjected to analyte concentration determination according to the invention. The analyte

concentration is PT. The determination is performed at ambient room temperature of 21.5°C.

Sample 1 and Sample 2 are analyzed 5 times and 6 times, respectively. Blood was picked

25   up, contacted and mixed with the PT reagent using a semi-quantitative device, a platineuse.

The volume of the blood was thus imprecisely allotted. The intended volume of reagent was

350 µL. The platineuse was also used in hooking to determine the clotting time. With Sample

2, the operation of picking up blood is purposefully done in a sloppy manner to induce a

greater variation of the blood volume, as may be experienced at a near-patient assay site.

30   The PT, by INR, of the anticoagulated plasma of the anticoagulated blood samples and their

hematocrits were known by an accurate laboratory method. The values were INR 1.00 and

55.3% for Sample 1, and INR 2.44 for Sample 2. The HCT values are needed for the

determination of PT of anticoagulated plasma, since the composition of the reaction mixture

was ill defined. The known INR values were for comparison only. Determination of PT in

35   anticoagulated plasma, according to the invention, requires two or more measurements on

the mixture of blood and reagent. In the example, these were two optic measurements and a

rheologic measurement. The latter yielded a clotting time. The optic measurement allowed

determination of an apparent hematocrit, HCTa, as detailed in Table 1. The rheologic measurement allowed the determination of a PT. The PT determination was calibrated with anticoagulated blood calibrators with known PT, by INR, by an accurate laboratory method. At the ambient temperature, the ISI is 1.17 and the NCT is 55.2 seconds. The mean

5   hematocrit of the calibrators was 37.1%. The analyte concentration of the cell volume of anticoagulated blood was assumed to be 29% of that of the plasma volume, as established in Example 2. The hypothetical analyte volume is thus the volume of anticoagulated blood multiplied by (1-HCT+0.29*HCT). The ratio of mean Vh of the calibrators, Vhm, and the Vh of Sample 1 is thus (1-0.368+0.29*0.368)/(1-0,553+0.29*0.553) or 1.23. The corresponding for

10 Sample 2 is 1.07. The PT by INR in postulated anticoagulated plasma is determined as follows. The apparent INR value, INRa, is re-expressed by PT%, by use of an equation of Lindahl et al, PT%=1/(INR*0.028-0.018). The true PT of the blood by PT%t is obtained by use of Equation 1. The PT of postulated citrate anticoagulated plasma is determined as PT%pcit=PT%t*Vhm/Vh. The value of the invention is clearly shown by the determinations

15 on Sample 2. In the determination, the blood volumes fluctuated considerably, and so did the apparent blood PT, (aINR±CV) was 4.21±37.1%. According to the invention the PT of postulated anticoagulated plasma is INR 2.43±2.2%. This is in good agreement with INR 2.44 by an accurate laboratory method. The PT of Sample 1 was also accurately and precisely determined according to the invention. The example is, in sense a mock-up, of a

20 practice of the invention. For reason of experimental simplicity, the blood samples tested were citrate anticoagulated blood. In authentic practice of the invention, blood would have been tested. Instead of 10 µL of citrate anticoagulated blood sample, 9 µL of blood would have been used. The reduced volume of blood is to compensate for the volume expansion caused by the citrate anticoagulation process. Alternatively the PT determination would have

25 been calibrated with 11.11 µL of citrate anticoagulated plasma and 10 µL of blood would have been tested. It is known that the citrate anticoagulation process, as such, does not influence the PT determination.

Example 3.

30   A set of 40 citrate anticoagulated blood samples were randomly selected from fully analyzed, to be discarded, samples, that during the previous day had been remitted to the Central Laboratory of the University Hospital Linköping for routine coagulation analysis. The samples were centrifuged so that the blood cells were pelleted at the bottom of blood sample tubes and the plasma was on top. Within two hours of analysis, the INR of the

35 plasma sample was determined by an accurate laboratory method of the central laboratory. The tubes were capped and turned upside down, several times, to resuspend the blood cells. Within 5 minutes of resuspension, each blood sample was analyzed, at room temperature,

21.5 C, by contacting10 µL (pipette) of blood with 350 µL of Owren PT reagent. Mixing and hooking was with a platineuse. Two measurements were performed on the mixtures, an optical with which the hematocrit, HCT, was determined and a rheological (the hooking) to determine the clotting time, CT. The set was subdivided into three groups. The first is a low

5   HCT group consisting of six samples with a mean HCT of 24.8%. The second is a high HCT group of eight samples with a mean HCT of 52,9%. The third is an intermediate HCT group of twenty-three samples with a mean HCT of 33.6%. The intermediate group is used to calibrate PT determination of the subject method. This resulted in an ISI of 1.11 and an NCT of 48.5 seconds. The mean plasma INR and blood INR of the low HCT group and the high

10   HCT group was 1.81 and 1.71, and 1.68 and 1.89, respectively. The blood INR compared to the plasma INR is 5.8% lower for the low HCT group and 12.5% higher in the high HCT group. To establish the size of the hypothetical analyte volume, Vh, the mean blood INR values for the high and the low HCT group is converted into PT%, a proportional PT expression. The mean plasma PT% of each group is used to determine a mean blood PT%

15   by multiplying the mean blood PT% with the quotient of the mean Vh of the calibrators and the mean Vh of the group. The Vh is assumed to be the sum of the plasma volume and a fraction of the cell volume of anticoagulated blood. The fraction is varied between 0 and 1 in steps of 0.1. At each fraction level the mean plasma INR is determined and compared to the known mean plasma INR. At a fraction of 0, all PT in the plasma volume, the plasma INR

20   was 2.5% greater and 9.1% smaller than the known plasma INR for the low HCT and the high HCT groups, respectively. A difference of zero is found at cell volume fractions of 0.24 and 0.33 for the low HCT group and the high HCT group, respectively. See Figure 2. The optimal value of the cell volume fraction is established at 0.29 (29%). Practice of the invention in determination of postulated plasma PT by analysis of blood PT leads to

25   considerable improvement in accuracy when hematocrit is high. At a hematocrit of 58.8% (equivalent to 52.9% in anticoagulated blood), practice of the invention at a smaller laboratory will erase a systematic error of 12% in determination of PT by analysis of blood according to prior art. At hematocrits of 55%, 60%, 65% and 70% the systematic error that will be erased by practice of the invention is 11%, 17%, 23% and 29%, respectively, in the

30   therapeutic INR range of 2.5. Systematic errors at low hematocrit, although somewhat more modest, will also be erased, by practice of the invention. In the example, for experimental reasons, 10 µL of citrate anticoagulated blood was added to the PT reagent, instead of 9 µL of blood, in practice of the invention. In this regard, the practice of the invention in this example is a mock-up. However, there is no reason to believe that the results would have

35   differed by use of blood.

Example 4.

     The same data set as in Example 3 is used to practice the invention by the procedural steps involving Equation 4. There are the same three groups as in Example 3; the low HCT group, the high HCT group and the intermediate HCT group. Using the cell volume
5   fraction established in Example 3, Vh of each sample is determined as 10*(1-HCT+0.29*HCT). The mean Vh, Vhm, for the low, high and intermediate HCT groups are 8.24 µL, 6.25 µL and 7.62 µL, respectively. Calibration of the PT determination was as in Example 3. Three plots of blood PT, INRb, against the known plasma PT, INRp, one for each group, are made, see Figure 3a. Linear least square regression yielded the regression
10   equations INRb=0.935*INRp+0.011 and INRb=1.108*INRp+0.021, for the low and high HCT group, respectively. From this a difference was calculated ΔINRb=-0.173*INRp+0.099. The corresponding difference in mean Vh, ΔVhm, was determined to 1.99 µL. The ΔINRb/ΔVhm, viewed as an estimate of dINRb/dVh, was thus established as -0.086*INRb+0.005. This was used to determine the plasma INR of the samples in the low HCT group and the high HCT
15   group according to Equation 11, see Figure 3b. The mean error, (INRb-INRp)/INRp, was determined for each sample before and after the correction. In the low HCT group the error changed form −4.8% to 0.2% and for the high CHT group from 12.1% to 0.8%. The here used procedure to practicing the invention in determine an analyte concentration by a non-proportional expression, the use of Equation 4, requires fewer, and simpler, calculations than
20   alterative procedure described previously, i.e. procedures in which an analyte concentration by a non-proportional is re-expressed into an analyte concentration by a proportional expression. The here described procedure, which requires less calculation, may be of advantage when practicing the subject methods by use of a microprocessor with limited processing capacity.

25

Example 5.

     The following is given to show how an analyte concentration in anticoagulated plasma may be determined according the invention. Particularly, it is an example of how the computation is performed. The analyte concentration of the example is prothrombin time, PT.
30   The analyte concentration is expressed in INR.

     Each member of a set of 59 blood samples, with known INR value of its corresponding anticoagulated plasma, as determined by an accurate laboratory method, was analyzed by a method according to the invention. At time zero, at ambient room temperature, 20 µL of blood sample was added to and mixed with 400 µL of PT reagent in a tubular
35   container of polystyrene, which was lodged in the container holder of a subject measuring and determination device. More than two different measurements were made on the mixture. The amount of light, from a 940 nm LED, transmitted through the mixture (I), the time at

which the mixture coagulated (CT) and the temperature of the mixture (t) were measured. The amount of light transmitted through the reagent alone, prior to addition of the blood, was also measured (Io). One of the different measurements, I, correlates with the hematocrit of the blood, as does I/Io, as is obvious to those skilled in the art and as shown in Example 1

5   and Table 1. One of the different measurements, CT, correlates with the PT of the anticoagulated blood, as is also obvious and is reflected by the data in Table 2. The temperature of the mixture, which was in thermal equilibrium with the environment, was measured by measuring the ambient room temperature. According to the invention, the PT of the anticoagulated blood is computed from the values of different measurements of the

10   mixture, i.e. it is computed by a mathematical function of I, CT and t, FI1(I, CT, t). PT of the anticoagulated plasma corresponding to each of the 59 plasma samples was computed, according to the invention, by a function FI((I/Io), CT, t). According to prior art the PT was computed as a function of only CT and t, FPA(CT, t). Good functions were arrived at by calibration with 2 anticoagulated plasma samples, calibrators, with known INR values set by

15   multi-centra exercises organized by the standardization organization EQUALIS, Uppsala, Sweden. The calibration was done at various temperatures in the range of 19 to 27°C. This allowed the Normal Clotting Time, NCT, and the International Sensitivity Index, ISI, to be determined as functions of temperature, NCT(t) and ISI(t), respectively. The time and temperature are in arbitrary units used internally by the computer, timeU and tempU,

20   respectively. One tmu is about 0.0665 seconds and tepu are converted into degrees centigrade (t) by the relationship t = 80.9 − 0.109*tempU. The conversions are for humans only and have no bearing on the computations. The functions were:

$$NCT(t) = -2329 - 9.547 * tempU + 0.0113 * tempU * tempU$$

25

$$ISI(t) = 0.8785 + 0,0007 * tempU$$

In the temperature range specified, NCT(t) was described by a second degree polynomial of computers temperature unit and ISI(t) as a first. In analyzing anticoagulated

30   plasma, the computation is straight forward:

$$INR = (CT/NCT(t))expISI(t).$$

By analyzing anticoagulated blood samples and corresponding anticoagulated

35   plasma samples, at various temperatures within the temperature range, it was found that NCT and ISI of blood had the temperature functions 1.17*NCT(t) and 0.775*ISI(t), respectively. The PT of an anticoagulated plasma can be determined by measuring the

cotting time, CT, of an admixture of citrate anticoagulated blood and liquid PT reagent. Accordingly, if anticoagulated blood is the sample, the INR of anticoagulated plasma, here called INRcb, because citrated blood is the sample, is:

$$INRcb = \{CT/[1.17*NCT(t)]\}exp[0.775*ISI(t)]$$

This was strictly valid only when the hematocrit of the anticoagulated blood was the average of that of the anticoagulated blood samples used to derive the expression.

If blood instead of anticoagulated blood is mixed with liquid reagent to the same proportion, 1.11 fold more blood sample is added, the effects on NCT and ISI can be computed using the concept of hypothetical volume and the relationship between PT% and INR which is described by Lindahl et al, as described above. The NCT will decrease to 95.4% and the ISI increased to 103.4%. Experimental determination using reagent volumes reduced by 10%, gave essentially the same results. Hence, the PT of anticoagulated plasma can be determined by measuring the clotting time of blood and PT reagent, according to the same protocol, according to the below. The INR of the anticoagulated plasma determined in this way is called INRb, and was:

$$INRb = [CT/1.116*NCT(t)]exp0.801*ISI(t)$$

Again, the PT of anticoagulated plasma, determined by mixing anticoagulated blood or blood, using the same protocol, was given by the relationships above, but only if the hematocrit was at the average level of the computation in question. According to the present invention, at least one measurement that correlates to hematocrit is made on the mixture of blood and reagent. The measurement of light transmitted through the mixture, I, is such a measurement. For technical reasons, variations in size and properties of the container, the container's positioning in the container holder, the energy sourcing of the light source, and foremost, individual differences between devices of a given lot of devices, it is better to use the quotient I/Io, than I, as a measure of the admixture that correlates with the hematocrit of the blood sample. To correct, according to the invention, for effects of variations of the hematocrit of the sample, the dependence of INR on the I/Io was established. To do this, the 59 INRcb obtained as described above, were expressed as a fraction of the known corresponding INR values for their anticoagulated plasma. The quotient was called K and ranged from 0.85 to 1.23 amongst the 59 samples. The dependence of K on I/Io, the measure of hematocrit, was deduced by linear regression and a significant correlation was found. The equation of the regression line was K = 0.032*(I/Io) + 0.745. This allowed · computation of the INRcbi according to the invention:

$$INRcbi = \{CT/[1.17^*NCT(t)]\}exp[0.775^*ISI(t)]/[0.032^*(I/Io)+0.745]$$

Inspection showed that the mean absolute deviation of the 59 PT

5   determinations from the known values was 8%, the maximal deviation observed was 23% for
the PT determined according to prior art. The corresponding values for PT determination
according to the invention were 6% and 14%. Obviously, determination according to the
invention resulted in a considerably improvement in accuracy, as compared to prior art.
From the above relationship, $K = 0.032^*(Io/I) + 0.745$, average hematocrit appears to be at

10   Io/I of 7.969. If blood had been the sample, the average hematocrit would have appeared at
8.846 and, hence, the relationship $K = 0.032^*(Io/I) + 0.717$. The following computation was,
hence, used in determination of the PT in anticoagulated plasma by performing two or more
measurements on an admixture of blood and liquid PT reagent, according to the invention.
The determined PT was called INRbi:

15

$$INRbi = \{CT/[1.116^*NCT(t)]\}exp[0.801^*ISI(t)]/[0.032^*(I/Io)+0.717]$$

The above examples are given to display how the present invention may be
practiced. Naturally, there are innumerable variations in how the measurements and

20   computations may be performed without departing from the scope of the invention which
provides methods, devices and kits for determining an analyte concentration in
anticoagulated plasma at many smaller medical centers where preparation of anticoagulated
plasma is inconvenient or impossible to prepare.

25

Tables

Table 1. Six EDTA anticoagulated samples, 1A through 6AS, with various hematocrit, HCT, are analyzed by an analytical set-up with which the invention may be practiced. The blood volume, Vb, is added to 350 μL of PT reagent. From one of the samples, sample 6AS, experiments with various volumes are performed. The normalized blood concentration is determined by use of Equation 3. A relationship between HCT and I/Io is established. With this relationship and the Io/I quotient, an apparent hematocrit is determined for each experiment, HCTd. By use of Equation 2, the added blood volume is determined, Vbd.

Sample	HCT	Vb	Io	I	HCTa	Vbd	Vbd/Vb%
1A	39.3	10	731	48	40.3	10.3	103
2A	47.7	10	757	47	42.1	8.8	88
3A	45.0	10	753	44	44.1	9.8	98
4A	25.1	10	742	110	23.1	9.2	92
5A	31.5	10	809	65	34.6	11.0	110
6AS	44.0	10	825	50	42.9	9.7	97
4S	17.9	4	797	142	20.8	4.6	116
6S	26.7	6	768	92	26.3	5.9	99
8S	35.4	8	806	62	35.8	8.1	101
12S	52.5	12	828	38	53.6	12.3	102
14S	60.9	14	808	30	64.0	14.7	105
16S	69.2	16	749	26	67.8	15.7	98
18S	77.5	18	739	22	77.6	18.0	100

Table 2. Near-patient practice of a method of the invention in which the analyte
concentration PT was determined 5 times and 6 times in two blood samples, Sample 1 and
Sample 2, respectively. By accurate laboratory methods, the plasma PT and HCT of Sample
1 and Sample 2 were INR 1.00 and 55.3% and INR 2.44 and 43.5%, respectively. Io and I
are light intensities transmitted through the reagent alone or through the mixture of blood and
reagent. CT is the clotting time. HCTa and INRa are the apparent hematocrit and PT,
respectively. PT%a is the apparent PT by PT%. PT%t is the true PT% of the blood. PT%p
and INRp are the PT in postulated anticoagulated plasma by PT% and by INR. The mean
and the CV of INRa and INRp are also given.

Sample 1

Io	I	CT	HCTa	INRa	PT%a	PT%t	PT%p	INRp
817	33	58	59.6	1.06	85.8	79.6	98.3	1.01
806	30	55	63.9	1.00	101.2	87.6	108.1	0.97
772	36	60	52.9	1.10	77.9	81.4	100.5	1.00
791	38	60	51.6	1.10	77.9	83.4	103.0	0.99
843	36	58	56.9	1.06	85.8	83.4	102.9	0.99
Mean				**1.06**				**0.99**
CV				**4.1%**				**1.2%**

Sample 2

Io	I	CT	HCTa	INRa	PT%a	PT%t	PT%p	INRp
787	43	120	46.5	2.46	19.6	18.4	19.7	2.46
824	44	120	47.4	2.46	19.6	18.0	19.3	2.49
753	45	122	43.4	2.51	19.1	19.2	20.6	2.38
794	65	147	34.2	3.11	14.4	18.4	19.7	2.45
850	147	193	21.1	4.27	9.8	20.3	21.7	2.29
817	38	111	53.0	2.25	22.2	18.3	19.6	2.47
Mean				**2.84**				**2.42**
CV				**26.6%**				**3.2%**